3. Please delete the paragraph beginning at line 15 on page 24 and replace it with the following paragraph:

Poly-G nucleic acids preferably are nucleic acids having the following formula:

5' $X_1X_2GGGX_3X_4$ 3'

wherein X_1, X_2, X_3, and X_4 are nucleotides. In preferred embodiments at least one of X_3 and X_4 is a G. In other embodiments both of X_3 and X_4 are G's. In yet other embodiments the preferred formula is 5' GGGNGGG 3' (SEQ ID NO:168) or 5' GGGNGGGNGGG 3' (SEQ ID NO:169), wherein N represents between 0 and 20 nucleotides. In other embodiments the poly-G nucleic acid is free of CpG dinucleotides, such as, for example, the nucleic acids listed in Table 4 as SEQ ID NOs 95-114, 117-121, 123-130, 132, and 133. In other embodiments the poly-G nucleic acid includes at least one CpG dinucleotide, such as, for example, the nucleic acids listed in Table 4 as SEQ ID NOs 115, 116, 122, 131, and 134-136. Particularly preferred ISNAs are SEQ ID NOs 134, 135, and 136.

Remarks

Claims 1-24, 47, 65, 82, 103, 122, 143, 159, 176, 199, and 201-203 are pending. Claims 47, 122, 143, 159, and 201 have been withdrawn from consideration following election in response to a Restriction Requirement made final by the Examiner. In response to the Office Action mailed August 28, 2002, Applicants have amended Claims 1, 19, 24, 65, 82, 103, 176, 199, and 202 and the Specification. No claims have been added. No claims have been cancelled. No new matter is introduced.

Sequence Compliance Objections

The Examiner objected to the disclosure because the Sequence Listing did not comply with the requirements of 37 C.F.R. §§ 1.821 – 1.825. Applicants have amended the specification at page 68, lines 25 and 26, to assign SEQ ID NOs to the two amino acid sequences pointed out by the Examiner. Applicants have also amended the specification at page 24, line19, to assign

SEQ ID NOs to two nucleic acid sequences. Applicants have also filed, under separate cover directed to United States Patent and Trademark Office, Box Sequence, P.O. Box 2327, Arlington, VA 22202, a Substitute Sequence Listing in paper and computer readable form. The Substitute Sequence Listing incorporates the two amino acid sequences and the two nucleic acid sequences assigned SEQ ID NOs by this Amendment. It is believed that the Substitute Sequence Listing complies with the requirements of 37 C.F.R. §§ 1.821 – 1.825.

Claim Objections

Claim 176 is amended so the abbreviations "IFN" and "IPCs" are written out at the first occurrence of the terms. Claims 19 and 202 have been amended so they no longer contain non-elected SEQ ID NOs. In particular, these claims have been amended to include only SEQ ID NOs 7, 9, 11, 13, 24, 25, 30, 33, 36 and 37. By amendment to Claim 202, Claim 203 no longer contains non-elected SEQ ID NOs. Accordingly, it is believed that the claim objections have been overcome by this Amendment.

Rejection Under 35 U.S.C. § 112, Paragraph 1 (Written Description)

The Examiner rejected claims 1-11, 17-18, 20-24, 65, 82, 103, 176, and 199 under 35 U.S.C. § 112, Paragraph 1, for alleged lack of sufficient written description. More particularly, the Examiner appears to object to an alleged lack of sufficient relevant identifying characteristics of the immunostimulatory nucleic acids which are elements of the claimed invention. Applicants have amended claims 1, 24, 65, 82, 103, 176, and 199 so as further to specify structural features of the immunostimulatory nucleic acids. In particular, the claim language is amended to specify the immunostimulatory nucleic acid is at least 8 nucleotides long and comprises a poly-G sequence at each end and a central palindromic sequence comprising an unmethylated CpG dinucleotide. Applicants assert that the specification amply teaches a representative number of species to support claims drawn to the broad genus of such immunostimulatory nucleic acid molecules. Specifically, at least the following SEQ ID NOs disclosed on pages 26-27 of the specification fall within the ambit of the nucleic acids described by the amended claims: 1 and 5-

37. Applicants thus respectfully request the Examiner to withdraw the rejection under 35 U.S.C. § 112, Paragraph 1 (written description), in view of the amendments to the claims.

Rejection Under 35 U.S.C. § 112, Paragraph 1 (Enablement)

The Examiner rejected claims 1-11, 17-18, 20-24, 65, 82, 103, 176, 199, and 202-203 under 35 U.S.C. § 112, Paragraph 1, for alleged lack of enablement. More particularly, the Examiner appears to object to an alleged lack of enablement for the use of any immunostimulatory nucleic acid in the practice of the invention.

Applicants submit that the amendments to the claims to overcome the Examiner's rejection under 35 U.S.C. § 112, first paragraph (written description) also overcome the enablement rejection. As amended, the claims are directed to immunostimulatory nucleic acids having specific structural features that provide the skilled artisan sufficient guidance to practice the full breadth of the claimed invention without undue experimentation. Namely, rather than being drawn to any immunostimulatory nucleic acid, the amended claims are drawn to immunostimulatory nucleic acids at least 8 nucleotides long and comprising a poly-G sequence at each end and a central palindromic sequence comprising an unmethylated CpG dinucleotide.

Applicants wish to point out to the Examiner that they do not intend to be bound by the Examiner's assertion, on page 13 of the Office Action, that "an immunostimulatory DNA sequence must contain at least unmethylated CpG dinucleotides in a consensus motif 5′-purine-purine-CpG-pyrimidine-pyrimidine-3′. Indeed, notwithstanding the Examiner's citations to U.S. Patents 6,194,388, 6,239,116, and 6,214,806, the instant invention clearly teaches that the particular class of immunostimulatory nucleic acids that induces IFN-α expression need not conform to the rule cited above. It will be appreciated, for example, that elected SEQ ID NOs 7, 9, 11, 13, 24, 25, and 33 do not conform to the 5′-purine-purine-CpG-pyrimidine-pyrimidine-3′ paradigm.

The Examiner also appears to object to an alleged lack of enablement for in vivo methods. In this regard, Applicants respectfully submit that the Examiner has misunderstood the application insofar as, contrary to the Examiner's reasoning expressed on pages 13-15 of the Office Action, the application is not directed to a form of gene therapy. As such, there is no need

to be concerned with "vector targeting" because the nucleic acids of the instant invention are not believed to require or even to involve expression of gene products encoded by the nucleic acids of the invention. Therefore, Applicants respectfully request the Examiner to withdraw the rejection as it concerns gene therapy, because the claimed invention is not directed to methods of gene therapy.

Further addressing the Examiner's apparent concerns about an alleged lack of enablement for in vivo methods, it will be appreciated that the administered immunostimulatory nucleic acids of the instant invention can include but do not necessarily require a modified nuclease-resistant backbone to effect the desired results in vivo. Indeed, both the presence of a palindromic sequence and of poly-G ends (which can form G-tetrads, for example), without more, would suggest that the immunostimulatory nucleic acids of the invention can have secondary structure that can stabilize them and increase their effect (see, e.g., page 28, lines 1-9). In addition, as described on pages 25-26 of the specification, and as is true of all the elected SEQ ID NOs, certain preferred immunostimulatory nucleic acids of the instant invention incorporate a chimeric, partially phosphate-modified backbone, e.g., a phosphodiester/phosphorothioate backbone. Such a backbone is also expected to be stabilized against nuclease degradation.

Applicants are puzzled by the Examiner's remarks on the bottom of page 15 of the Office Action, to the effect that with respect to composition claims 202-203, the specification fails to provide sufficient guidance for a skilled artisan on <u>how to use</u> the claimed composition [emphasis as supplied]. Applicants submit that the said composition claims stand on their own. In any case, for reasons given above, the observations by the Examiner vis-à-vis gene therapy and an alleged requirement of a modified nuclease-resistant backbone appear misplaced.

For the foregoing reasons, Applicants respectfully request the Examiner to withdraw the rejection under 35 U.S.C. § 112, Paragraph 1 (enablement).

<u>Rejection Under 35 U.S.C. § 112, Paragraph 2</u>

The Examiner rejected claims 4-7, 20-23, and 103 under 35 U.S.C. § 112, Paragraph 2. Claims 4-7, 20-23, and 103 are amended to more particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Claims 4-7 and 20-23, as they depend

from claim 1 as amended to include "the subject", no longer lack antecedent basis for this claim term. The preamble of Claim 103 has been amended to substitute "reducing" for "preventing". Applicants respectfully request the Examiner to withdraw the rejection under 35 U.S.C. § 112, Paragraph 2, in view of the above claim language amendments.

Summary

Claims 1, 19, 24, 65, 82, 103, 176, 199, and 202 have been amended to overcome all the objections and rejections raised by the Examiner in the Office Action. A Substitute Sequence Listing is filed concurrently with this Amendment, under separate cover.

Applicants believe the claims are in condition for allowance. An early and favorable response is earnestly solicited.

Respectfully submitted,

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Docket No.: C01039.70044.US
Dated: February 11, 2003
XD 02/28/03

Marked-Up Versions of Amended Claims

1. (amended) In a method which calls for administration of IFN-α to a subject, the improvement comprising co-administering an effective amount of an isolated immunostimulatory nucleic acid, wherein said isolated immunostimulatory nucleic acid is at least 8 nucleotides long and comprises a poly-G sequence at each end and a central palindromic sequence comprising an unmethylated CpG dinucleotide.

19. (amended) The improvement of claim 1, wherein the immunostimulatory nucleic acid has a sequence selected from the group consisting of

[ggGGTCAACGTTGAgggggG	ODN 1585	SEQ ID NO:1
tcgtcgttttgtcgttttgtcgtt	ODN 2022	SEQ ID NO:2
ggggtcgtcgttttgggggg	ODN 2184	SEQ ID NO:3
tcgtcgttttgtcgttttgggggg	ODN 2185	SEQ ID NO:4
ggggtcgacgtcgaggggggg	ODN 2192	SEQ ID NO:5
ggggtcatcgatgaggggggg	ODN 2204	SEQ ID NO:6]
ggGGGACGATCGTCgggggG	ODN 2216	SEQ ID NO:7
[gggggtcgtacgacgggggg	ODN 2217	SEQ ID NO:8]
ggGGGACGATATCGTCgggggG	ODN 2245	SEQ ID NO:9
[ggGGGACGACGTCGTCgggggG	ODN 2246	SEQ ID NO:10]
ggGGGACGAGCTCGTCgggggG	ODN 2247	SEQ ID NO:11
[ggGGGACGTACGTCgggggG	ODN 2248	SEQ ID NO:12]
ggGGGACGATCGTTGgggggG	ODN 2252	SEQ ID NO:13
[ggGGAACGATCGTCgggggG	ODN 2253	SEQ ID NO:14
ggGGGGACGATCGTCgggggG	ODN 2254	SEQ ID NO:15
ggGGGACGATCGTCGgggggG	ODN 2255	SEQ ID NO:16
ggGGGTCATCGATGAgggggG	ODN 2260	SEQ ID NO:17
ggGGTCGTCGACGAgggggG	ODN 2293	SEQ ID NO:18
ggGGTCGTTCGAACGAgggggG	ODN 2294	SEQ ID NO:19
ggGGACGTTCGAACGTgggggG	ODN 2295	SEQ ID NO:20
ggGGAACGACGTCGTTgggggG	ODN 2297	SEQ ID NO:21
ggGGAACGTACGTCgggggG	ODN 2298	SEQ ID NO:22
ggGGAACGTACGTACGTTgggggG	ODN 2299	SEQ ID NO:23]
ggGGTCACCGGTGAgggggG	ODN 2300	SEQ ID NO:24
ggGGTCGACGTACGTCGAgggggG	ODN 2301	SEQ ID NO:25
[ggGGACCGGTACCGGTgggggG	ODN 2302	SEQ ID NO:26
ggGTCGACGTCGAgggggG	ODN 2303	SEQ ID NO:27
ggGGTCGACGTCGagggg	ODN 2304	SEQ ID NO:28
ggGGAACGTTAACGTTgggggG	ODN 2305	SEQ ID NO:29]

ggGGACGTCGACGTggggG	ODN 2306	SEQ ID NO:30
[ggGGGTCGTTCGTTgggggG	ODN 2311	SEQ ID NO:31
ggGACGATCGTCGgggggG	ODN 2328	SEQ ID NO:32]
ggGTCGTCGACGAgggggggG	ODN 2329	SEQ ID NO:33
[ggTCGTCGACGAGgggggG	ODN 2330	SEQ ID NO:34
ggGGACGATCGTCGgggggG	ODN 2332	SEQ ID NO:35]
ggGGTCGACGTCGACGTCGAGgggggG	ODN 2334	SEQ ID NO:36, and
ggGGACGACGTCGTGgggggG	ODN 2336	SEQ ID NO:37,

wherein each lower case letter represents phosphorothioate linkage and each upper case letter indicates phosphodiester linkage.

24. (amended) A method of supplementing IFN-α treatment of a subject, comprising administering to a subject in need of IFN-α treatment an effective amount of IFN-α and an isolated immunostimulatory nucleic acid, wherein said isolated immunostimulatory nucleic acid is at least 8 nucleotides long and comprises a poly-G sequence at each end and a central palindromic sequence comprising an unmethylated CpG dinucleotide.

65. (amended) A method of increasing efficacy of IFN-α treatment of a subject, comprising:

administering to a subject in need of treatment with IFN-α a pharmaceutical composition comprising IFN-α, and

coadministering to the subject in need of such treatment a pharmaceutical composition comprising an immunostimulatory nucleic acid in an amount which, together with the administered IFN-α, is an effective IFN-α treatment, wherein the efficacy of the IFN-α treatment is greater than the efficacy of administering the same amount of IFN-α in the absence of coadministering the immunostimulatory nucleic acid, and wherein said immunostimulatory nucleic acid is at least 8 nucleotides long and comprises a poly-G sequence at each end and a central palindromic sequence comprising an unmethylated CpG dinucleotide.

82. (amended) A method of decreasing a dose of IFN-α effective for treating a subject, comprising:

administering to a subject in need of treatment with IFN-α a pharmaceutical composition comprising IFN-α, and

coadministering to the subject in need of such treatment a pharmaceutical composition comprising an immunostimulatory nucleic acid in an amount which, together with the administered IFN-α, is an effective IFN-α treatment, [and,] wherein the amount of administered IFN-α is less than an amount of IFN-α required in the absence of coadministering the immunostimulatory nucleic acid, and wherein said immunostimulatory nucleic acid is at least 8 nucleotides long and comprises a poly-G sequence at each end and a central palindromic sequence comprising an unmethylated CpG dinucleotide.

103. (amended) A method of reducing [preventing] an IFN-α treatment-related side effect in a subject receiving or in need of treatment with IFN-α, comprising

administering to a subject in need of treatment with IFN-α a pharmaceutical composition comprising IFN-α, and

coadministering to the subject in need of such treatment a pharmaceutical composition comprising an immunostimulatory nucleic acid in an amount which, together with the administered IFN-α, is an effective IFN-α treatment, [and,] wherein an IFN-α treatment-related side effect is reduced in comparison to the side effect when IFN-α is administered in the absence of coadministering the immunostimulatory nucleic acid, and wherein said immunostimulatory nucleic acid is at least 8 nucleotides long and comprises a poly-G sequence at each end and a central palindromic sequence comprising an unmethylated CpG dinucleotide.

176. (amended) A method of stimulating production of a plurality of type I interferon (IFN) subtypes, comprising contacting type I interferon producing cells (IPCs) with an amount of immunostimulatory nucleic acid effective to induce secretion of at least two type I

interferons, wherein said immunostimulatory nucleic acid is at least 8 nucleotides long and comprises a poly-G sequence at each end and a central palindromic sequence comprising an unmethylated CpG dinucleotide.

199. (amended) A method of inhibiting IL-12 production, comprising

contacting IL-12-producing cells, in the presence of interferon-producing cells under conditions in which the IL-12-producing cells normally produce IL-12, with an immunostimulatory nucleic acid in an amount effective for inducing secretion of type I interferon, wherein said immunostimulatory nucleic acid is at least 8 nucleotides long and comprises a poly-G sequence at each end and a central palindromic sequence comprising an unmethylated CpG dinucleotide.

202. (amended) A pharmaceutical composition, comprising

an isolated nucleic acid having a sequence selected from the group consisting of:

[tcgtcgttttgtcgttttgtcgtt	ODN 2022	SEQ ID NO:2
ggggtcgtcgttttgggggg	ODN 2184	SEQ ID NO:3
tcgtcgttttgtcgttttgggggg	ODN 2185	SEQ ID NO:4
ggggtcgacgtcgaggggggg	ODN 2192	SEQ ID NO:5
ggggtcatcgatgaggggggg	ODN 2204	SEQ ID NO:6]
ggGGGACGATCGTCgggggG	ODN 2216	SEQ ID NO:7
[gggggtcgtacgacgggggg	ODN 2217	SEQ ID NO:8]
ggGGGACGATATCGTCgggggG	ODN 2245	SEQ ID NO:9
[ggGGGACGACGTCGTCgggggG	ODN 2246	SEQ ID NO:10]
ggGGGACGAGCTCGTCgggggG	ODN 2247	SEQ ID NO:11
[ggGGGACGTACGTCgggggG	ODN 2248	SEQ ID NO:12]
ggGGGACGATCGTTGgggggG	ODN 2252	SEQ ID NO:13
[ggGGAACGATCGTCgggggG	ODN 2253	SEQ ID NO:14
ggGGGGACGATCGTCgggggG	ODN 2254	SEQ ID NO:15
ggGGGACGATCGTCGgggggG	ODN 2255	SEQ ID NO:16
ggGGGTCATCGATGAgggggG	ODN 2260	SEQ ID NO:17
ggGGTCGTCGACGAgggggG	ODN 2293	SEQ ID NO:18
ggGGTCGTTCGAACGAgggggG	ODN 2294	SEQ ID NO:19
ggGGACGTTCGAACGTgggggG	ODN 2295	SEQ ID NO:20
ggGGAACGACGTCGTTgggggG	ODN 2297	SEQ ID NO:21
ggGGAACGTACGTCgggggG	ODN 2298	SEQ ID NO:22
ggGGAACGTACGTACGTTgggggG	ODN 2299	SEQ ID NO:23]

ggGGTCACCGGTGAggggggG	ODN 2300	SEQ ID NO:24
ggGGTCGACGTACGTCGAggggggG	ODN 2301	SEQ ID NO:25
[ggGGACCGGTACCGGTgggggG	ODN 2302	SEQ ID NO:26
ggGTCGACGTCGAggggggG	ODN 2303	SEQ ID NO:27
ggGGTCGACGTCGagggg	ODN 2304	SEQ ID NO:28
ggGGAACGTTAACGTTgggggG	ODN 2305	SEQ ID NO:29]
ggGGACGTCGACGTggggG	ODN 2306	SEQ ID NO:30
[ggGGGTCGTTCGTTgggggG	ODN 2311	SEQ ID NO:31
ggGACGATCGTCGgggggG	ODN 2328	SEQ ID NO:32]
ggGTCGTCGACGAgggggggG	ODN 2329	SEQ ID NO:33
[ggTCGTCGACGAGgggggG	ODN 2330	SEQ ID NO:34
ggGGACGATCGTCGgggggG	ODN 2332	SEQ ID NO:35]
ggGGTCGACGTCGACGTCGAGgggggG	ODN 2334	SEQ ID NO:36, and
ggGGACGACGTCGTGgggggG	ODN 2336	SEQ ID NO:37,

wherein each lower case letter represents phosphorothioate linkage and each upper case letter indicates phosphodiester linkage; and

a pharmaceutically acceptable carrier.

Marked-Up Version of Replacement Paragraph Beginning at Line 15, Page 24:

Poly-G nucleic acids preferably are nucleic acids having the following formula:

5' $X_1X_2GGGX_3X_4$ 3'

wherein X_1, X_2, X_3, and X_4 are nucleotides. In preferred embodiments at least one of X_3 and X_4 is a G. In other embodiments both of X_3 and X_4 are G's. In yet other embodiments the preferred formula is 5' GGGNGGG 3' (SEQ ID NO:168) or 5' GGGNGGGNGGG 3' (SEQ ID NO:169), wherein N represents between 0 and 20 nucleotides. In other embodiments the poly-G nucleic acid is free of CpG dinucleotides, such as, for example, the nucleic acids listed in Table 4 as SEQ ID NOs 95-114, 117-121, 123-130, 132, and 133. In other embodiments the poly-G nucleic acid includes at least one CpG dinucleotide, such as, for example, the nucleic acids listed in Table 4 as SEQ ID NOs 115, 116, 122, 131, and 134-136. Particularly preferred ISNAs are SEQ ID NOs 134, 135, and 136.

Marked-Up Version of Replacement Paragraph Beginning at Line 20, Page 68:

CD8+ T cells (1×10^6) from HLA A2 positive healthy donors were stimulated in 24 well plates in the presence or absence of CpG ODN 2006 (SEQ ID NO:147), 1585 (SEQ ID NO:1), or 2216 (SEQ ID NO:7) at 6 μg/ml with either a HLA A2-restricted peptide derived from the influenza matrix protein (GILGFVFTL; SEQ ID NO:166) or a peptide derived from the melan A/mart-1 protein (ELAGIGILTV; SEQ ID NO:167). Autologous PBMC (3×10^6) were used as APCs. After 14 days cells were harvested, washed, and restimulated with influenza matrix or melan-A peptides for 6 hours. Brefeldin A was added for the last 4 hours. Cells were stained for CD8 and CD3, subsequently fixed, permeabilized and stained with mAb against IFN-γ. Also after 14 days the percentage of tetramer-positive $CD8^+$ T cells (HLA-A2/melan-A-peptide and HLA-A2/influenza matrix-peptide) was determined by flow cytometry. Tetramers are fluorochrome-labeled MHC-peptide tetramers which are designed to bind specifically to a peptide-specific T cell receptor, making it possible to identify peptide-specific T cells using flow cytometry. Altman JD et al. *Science* 274:94-96 (1996); U.S. Patent No. 5,635,363.